EXPRESSION AND PURIFICATION OF PROTEINS FOR STUDYING MACROMOLECULAR CROWDING EXPERIMENTS, Tripta P Mishra, Apratim Dhar, Martin Gruebele*, University of Illinois at Urbana-Champaign, Department of Chemistry, Urbana, IL 61801, gruebele@scs.uiuc.edu

Protein folding has been traditionally studied in dilute buffers *in vitro*, typically at concentrations less than 10 g/L. However, in living cells, proteins fold under very different conditions, with solute concentrations being as high as 200 - 400 g/L. This high concentration of biological macromolecules is referred to as macromolecular crowding and is predicted to have significant effects on the stability and dynamics of proteins. One way to study the effect of crowding on folding is to put a low-melting protein in a matrix of a high-melting protein. For this purpose, this project aimed at expressing and purifying large quantities of Sub L, the matrix protein. High level expression of Sub L in E. coli was achieved, and a heat purification scheme was used in conjunction with affinity chromatography to obtain very high-purity protein. Low-resolution electrospray-ionization mass spectrometry and fluorescence measurements were used to confirm the purity of the protein. Preliminary crowding results obtained by Sharlene Denos show that a low-melting protein embedded in Sub L indeed undergoes a shift in stability. However, Sub L itself undergoes precipitation at high temperatures and high concentrations, thus limiting its applicable temperature range.